

### Amendment to the Specification

Please amend the specification as follow:

On page 16, replace paragraph 0041 with the following paragraph 0041.

[0041] On the day of transformation, 1  $\mu$ l of 20 mM acetosyringone was added per ml of NT-1 culture. The acetosyringone stock was made in ethanol the day of the transformation. The NT-1 cells were wounded to increase the transformation efficiency. For wounding, the suspension culture was drawn up and down repeatedly (20 times) through a 5 ml wide-bore sterile pipet. Four milliliters of the suspension was transferred into each of 10, 60 x 15 mm Petri plates. One plate was set aside to be used as a non-transformed control. Approximately, 50 to 100  $\mu$ l of *Agrobacterium* suspension was added to each of the remaining 9 plates. The plates were wrapped with ~~parafilm~~ PARAFILM® then incubated in the dark on a shaker at 100 rpm at  $25 \pm 1^{\circ}\text{C}$  for 3 days.

Beginning on page 16, replace paragraph 0042 with the following paragraph 0042.

[0042] Cells were transferred to a sterile, 50 ml conical centrifuge tube, and brought up to a final volume of 45 ml with NTC medium (NT-1 medium containing 500 mg/L carbenicillin, added after autoclaving). They were mixed, then centrifuged at 1000 rpm for 10 min in a centrifuge equipped with a swinging bucket rotor. The supernatant was removed, and the resultant pellet was resuspended in 45 ml of NTC. The wash was repeated. The suspension was centrifuged, the supernatant was discarded, and the pellet was resuspended in 40 ml NTC. Aliquots of 5 ml were plated onto each Petri plate (150 x 15 mm) containing NTCB10 medium (NTC medium solidified with 8g/l Agar/Agar; supplemented with 10 mg/l bialaphos, added after autoclaving). Plates were wrapped with ~~parafilm~~ PARAFILM® then maintained in the dark at  $25 \pm 1^{\circ}\text{C}$ . Before transferring to the culture room, plates were left open in the laminar flow hood to allow excess liquid to evaporate. After 6 to 8 weeks, putative transformants appeared. They were selected and transferred to fresh NTCB5 (NTC medium solidified with 8g/l Agar/Agar;

supplemented with 5 mg/l bialaphos, added after autoclaving). The plates were wrapped with ~~parafilm~~ PARAFILM® and cultured in the dark at  $25 \pm 1^\circ\text{C}$ .

Beginning on page 18, replace paragraph 0046 with the following paragraph 0046.

**[0046]** Transformed NT-1 cells were maintained as callus on agar plates prepared from NT-1 media containing 0.8% agar. The components of the media include 2.5 mM MES, 1.2 mM dibasic potassium phosphate (Lot No. 47HO811), 0.1% (w/v) myoinositol (Lot No.49H039025), 0.001% (w/v) thiamine HCl (Lot No.107H02785), 0.4% (w/v) MS salts (Lot No.470803), 3% (w/v) sucrose (Lot No.47H0803), and 0.8% (w/v) agar-agar (L#390906), all from Sigma, and 0.22% (v/v) 2,4-D (Lot No.107091) from Gibco. Suspension cells as well as agar plates also contained 50ug/ml of kanamycin (L#129HO8941, Sigma) as required to evaluate the selection for the NT-1 transformed recombinant DNA. Callus was passed by taking a sterile pipette to break the callus and transferring a small amount of the callus ( $0.5\text{ cm}^3$ ) to a fresh plate. To produce suspension cultures of the NT-1 cells, the callus was broken with a pipette and several pieces of the callus were transferred to NT-1 media in an Erlenmeyer flask, without the agar, and placed in a gyratory incubator at  $28\text{-}30^\circ\text{C}$ . Cells were harvested by several methods 6-12 days after passage. Whole wet cells were obtained by holding the shaker flask stationary to allow cells to settle, followed by decanting the media. Sonicated whole wet cells were obtained as described above by resuspending the wet cells in extraction buffer containing 50 mM sodium ascorbate, 1mM EDTA, 1mM PMSF, and 0.1% ~~Friton X-100~~ TRITON® X-100 (all from Sigma), prepared in phosphate buffered saline pH 7.2. The cells were then broken open using a Branson 450 sonifier with a flat replaceable tip at output control of 8, duty cycle 60 for 10 minutes on ice. Supernatant and pellet from sonicated wet cells were prepared by centrifuging the sonicated preparation 20-30 min at 3400 rpm using a Beckman GPR centrifuge. Whole dried cells were prepared by filtering whole wet cells using a Buchner funnel lined with Spectramesh; the packed cells were spread onto a Spectramesh sheet in a shallow plastic tray, then placed in a food dehydrator overnight.

Beginning on page 20, replace paragraph 0049 with the following paragraph 0049.

**[0049]** Nunc Maxisorp 96-well microtiter ELISA plates were coated with 5 ug/well of mixed GM1 ganglioside in 0.01 M borate buffer using 100 ul per well; plates were incubated at room temperature overnight. The plates were washed 3 times with PBS-~~Tween~~ TWEEN<sup>®</sup> (1X containing 0.05% ~~Tween~~ TWEEN<sup>®</sup> 20, Sigma Lot No.120K0248). Each well was then incubated one hour at 37°C with 200 ul of blocking buffer containing 5% (w/v) non-fat dried milk in PBS- 0.05% ~~Tween~~ TWEEN<sup>®</sup> 20. The wells were washed 1X with 250 ul/well using PBS-~~Tween~~ TWEEN<sup>®</sup> 20. Reference antigen and sample antigens were mixed with blocking buffer before adding to plates. LT reference antigen and LT-B reference antigen were diluted to 50ng/ml in the first well while samples were pre-diluted at several different starting dilutions. Samples were added to the plate by applying 200ul of sample in row A and 100 ul of blocking buffer to remainder rows. Mixing and transferring 100ul per well made serial 2-fold dilutions. Plates were then incubated 1h at 37°C, washed 3X in PBS-~~Tween~~ TWEEN<sup>®</sup> and 100ul of diluted antisera in blocking buffer was added per well and incubated 1h at 37°C. The plates were washed 3X in PBS-~~Tween~~ TWEEN<sup>®</sup> and then 100ul of antibody conjugate was added and incubated 1h at 37°C. The plates were washed 3X in PBS-~~Tween~~ TWEEN<sup>®</sup> and 50ul of TMB substrate was added to each plate and TMB stop solution was added at 20 minutes post addition of substrate. Optical density at 450 nm wavelength was determined using a Tecan Sunrise Plate reader. Data were transported and displayed using Tecan Magellan Software. Linear regression and quantitation analyses were done using Microsoft Excel 2000 version 9.0.3821 SR-1.

Beginning on page 22, replace paragraph 0053 with the following paragraph 0053.

**[0053]** Y1 adrenal cells from mice were purchased from ATCC (CCL-79, L#1353400). The cell vial was thawed at 37°C and placed into a 25 cm<sup>2</sup> T-flask (Corning) containing 10 ml of growth media consisting of 15% donor horse serum (Quad-5 L# 2212), 2.5% fetal bovine serum (JRH L# 7N2326), 1% ~~glutamax-1~~

GLUTAMAX-1™ (Gibco L# 1080323) in F-12K media (Gibco L# 1089716). Cells were incubated at 37°C in 5% CO<sub>2</sub>. Cells were maintained in this growth media at each passage and for LT and CT cytotoxicity assays. To assay, the cells are passed onto 96 well cell culture plates (Nunc) and allowed to reach 80% confluence. LT or CT toxin is diluted to 1ug/ml in F-12K growth media. The toxin is further diluted by two fold serial dilutions on a 96 well microtiter plate by adding 100ul of the prediluted sample to row A of the plate. Two fold serial dilutions are then made by transferring 50 ul of the sample in row A to 50 ul of growth media in the next well. Each dilution of the sample is transferred to 1-4 wells of Y1 adrenal cells depending on availability of samples or cells. The end point titer of CT or LT toxin is the ug/ml required to obtain 50% cytotoxicity (cell death). Guidry, J. J., Cardenas, L., Cheng, E. and J. D. Clements. 1993. Role of receptor binding in toxicity , immunogenicity and adjuvanticity of Escherichia coli heat-labile enterotoxin. Inf. and Imm. 65: 4943-4950.

On page 33, replace paragraph 0065 with the following paragraph 0065.

**[0065]** The included vaccine inoculations were done on days 0 and 14 of the study. Extracts from CHN18 were given at 7 ug at day 0, and 18 ug at day 14. *E. coli*-derived LT was mixed with the CHN18 extract using 8 ug at day 0 and 20 ug at day 14, and the plant-derived heat labile toxin (SLT 102) was given at 0.5 ug at day 0 and 1.5 ug at day 14. Samples treated with LT or SLT were compared to treatment groups receiving a more conventional water in oil adjuvant, which were prepared by resuspending the freeze dried antigen preparation in a final concentration of 2.5% Drakeol Oil containing 0.1.65% Span 80 in DPBS with 0.5% ~~Tween~~ TWEEN® 80. Samples were mixed using two syringes and a three-way stopcock to allow suspension of the antigen in the water and oil mixture.